EFFECT OF COPPER-SALINITY INTERACTION ON PROLINE AND SOLUBLE SUGARS CONTENTS IN RADISH (RAPHANUS SATIVUS L.)

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Abstract

Soil degradation by salinity and heavy metal pollution is a serious environmental problem. This work consists to determine the action of Copper and NaCl on Raphanus sativus L.; a suitable plant for eco-toxicological investigations. Stress is applied for two weeks after 45 days of Radish culture. The parameters analyzed; proline and soluble sugars are measured by a UV-Visible Spectrophotometer JENWAY6505. The results obtained reveal an increase in proline and sugars as a function of NaCl and copper-NaCl interaction increase, particularly in the aerial system and a decrease in these as Copper increases. The highest levels of proline (0.143Mg/g DM to 80 Meq/L) and sugars (2.73 Mg/g DM in the control) are recorded at the aerial part of Radish. From these results, it appears that salinity improves tolerance to metallic stress in the aerial part of Radish by increasing proline and sugars contents, which shows that salinity promotes phytoextraction and transport of copper to these aerial parts.

Key words: Raphanus sativus, Copper, NaCl, Proline, Sugars.

Introduction

The presence of heavy metals in saline environments is an ever more important problem due to the increasing pollution of saline soils by metallic elements. The highest salinity levels are found in arid and semi-arid regions. Agricultural land is polluted by heavy metals through the irrational use of fertilizers and other plant protection methods (Valentina et al., 2010). Copper is a potentially phytotoxic trace element in high concentration. It’s mainly manifested through rhizotoxicity and sometimes by Fe deficiency induction (Kabata-Pendias and Pendias, 1992; Marschner, 1995).

The majority of plant species accumulate some organic solutes (sugars, alcohol, proline) in response to stress. These organic solutes are called osmoprotectants because they don’t interfere with enzymatic activities even at high concentrations (Chen et al., 2007; Tavakkoli et al., 2012). Proline plays multiple roles in plant tissues exposed to abiotic stresses such as nutritional reserve for growth, protein and membrane stabilization, osmoprotection and free radical scavenging (Zouari et al., 2016). Soluble sugars are indicators of stress levels, because of its significant increase during severity; metabolic sugars allow resisting different stresses (Zerrad et al., 2006). They also appear to play an important role in maintaining turgidity pressure which is the basis of the different processes controlling a plant’s life (Hare et al., 1998).

The plant material used in this study is Radish (Raphanus sativus L.) from the Brassicaceae family, the plant was chosen for its use in the laboratory as a model plant for eco-toxicology studies of different pollutants (Sun et al., 2010), as well as for its better germination rate, rapid growth and high biomass (Bitour, 2012).

The objective of this work is to study the behaviour of Radish under various stresses; metallic (Copper), saline (NaCl) and the interaction between both stresses, by estimation of osmoregulatory contents; proline and soluble sugars and determination of the action of salinity on Radish response to copper stress.

Materials and Methods

Plant material and culture method

Radish culture was carried out in a controlled greenhouse at the Mostaganem University Experimental
Farm during winter 2017. The seeds of *Raphanus sativus* L. of the National variety are disinfected with 2% sodium hypochlorite for 10 min and then washed several times in distilled water. Then, they are germinated in alveoli containing compost for 15 days before being transplanted into cylinders of 30 cm high and 20 cm in diameter for 45 days, these cylinders contain a mixture of sand and compost with proportions of (2V/V). Watering was done 3 times a week, twice with distilled water and once with a nutrient solution of Hoagland (1938). After 60 days of culture in the alveoli and cylinders, Radish plants are harvested and transported to the laboratory to be cut into two aerial and root parts, then dried at 80°C for 48h (Neggaz and Reguieg Yssaad, 2018).

**Application of stress**

Radish plants are exposed to abiotic stresses twice for two weeks, the doses used in copper stress are (0, 400, 800 and 1000 PPM) and the doses used in NaCl stress are (0, 40, 80 Meq/L), as well as an interaction between these two stresses has been applied on Radish plants.

**Parameters analyzed**

The parameters analyzed in this work are proline and soluble sugars. The method used to determine the proline is that of (Troll and Lindsley, 1955) modified by (Dreier and Goring, 1974) and then by (Monneveux and Nemmar, 1986) and the determination of soluble sugars is made by the method of (Dubois, 1956). The instrument used to measure these parameters is the uv-visible spectrophotometer JENWAY 6505.

**Statistical analyses**

The software chosen to analyze the results obtained is the STATBOX software Version 6.4. The statistical analyses performed are the variance (ANOVA) and the NEWMAN-KEULS test with a significance level (P=5%).

**Results and Discussion**

**Effect of copper-salinity interaction on proline content**

The quantitative study of the proline content of Radish exposed to increasing concentrations of NaCl, reveals a significant increase in proline in both aerial and root parts of Radish, these levels are higher than those observed in the control. By contrast, the treatment with Cu causes a significant decrease in proline in the both parts of Radish (Table 2, Fig. 1). When Cu is combined with NaCl, root proline levels decrease proportionally with increasing metal and salt doses, but these levels remain higher in the Cu-80Meq/L interaction than Cu-40 Meq/L and Cu treatment. However, aerial proline concentrations decrease in the 800 PPM combined with 40 Meq/L. Beyond this dose, the proline content starts to increase gradually with the increase of Cu-NaCl doses (Table 2, Fig. 1).

Statistical analyses indicate that NaCl and Cu effect on the aerial and root proline levels of Radish is highly significant (P<0.005) and the effect of the Cu-NaCl interaction is not significant on the root proline content (P>0.05), but highly significant on its aerial content (P<0.005).

The proline content of *Raphanus sativus* L. decreases gradually with increasing doses of copper. These results are in accordance with several researchers showing a decrease in proline content in *Raphanus sativus* L. stressed by zinc (Tihana et al., 2008), *Brassica juncea* exposed to lead and cadmium stress (Jhon et al., 2009). The accumulation of proline in plants exposed to heavy metal pollution has been examined in several species, like *Cicer arietinum* L. (Tantrey and Agnihotri, 2010), *Rosa hybrida* L. (Kumar et al., 2010), *Pinus sylvestris* L. (Kandziora-Ciupa et al., 2016). Overall, the sensitive species accumulate proline more quickly. However, tolerant species, present a relative stability or low proline accumulation (Lemziri et al., 2007). Brinis and Belkhoudjia (2015) justified the reduced proline levels in stressed plants by the stress level that does not appear to have triggered the proteolysis necessary to obtain a high quantity of proline, therefore there

**Table 1:** Proline content (Mg/g Dry Matter) in Radish (*Raphanus sativus* L.) under copper (PPM) and NaCl (Meq/L) interaction.

<table>
<thead>
<tr>
<th>Copper NaCl</th>
<th>0</th>
<th>400</th>
<th>800</th>
<th>1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aarial part</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.065</td>
<td>0.054</td>
<td>0.037</td>
<td>0.031</td>
</tr>
<tr>
<td>40</td>
<td>0.14</td>
<td>0.049</td>
<td>0.028</td>
<td>0.037</td>
</tr>
<tr>
<td>80</td>
<td>0.143</td>
<td>0.035</td>
<td>0.038</td>
<td>0.056</td>
</tr>
<tr>
<td>0</td>
<td>0.079</td>
<td>0.059</td>
<td>0.05</td>
<td>0.046</td>
</tr>
<tr>
<td>40</td>
<td>0.086</td>
<td>0.066</td>
<td>0.04</td>
<td>0.042</td>
</tr>
<tr>
<td>80</td>
<td>0.1</td>
<td>0.091</td>
<td>0.069</td>
<td>0.065</td>
</tr>
</tbody>
</table>

**Table 2:** The soluble sugars content (Mg/g Dry Matter) in Radish (*Raphanus sativus* L.) under copper (PPM) and NaCl (Meq/L) interaction.

<table>
<thead>
<tr>
<th>Copper NaCl</th>
<th>0</th>
<th>400</th>
<th>800</th>
<th>1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aarial part</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2.73</td>
<td>2.479</td>
<td>1.484</td>
<td>1.378</td>
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<tr>
<td>40</td>
<td>2.529</td>
<td>2.382</td>
<td>1.987</td>
<td>1.254</td>
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<tr>
<td>80</td>
<td>2.703</td>
<td>2.108</td>
<td>1.532</td>
<td>2.19</td>
</tr>
<tr>
<td>Root part</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.877</td>
<td>1.758</td>
<td>1.408</td>
<td>1.359</td>
</tr>
<tr>
<td>40</td>
<td>2.248</td>
<td>1.876</td>
<td>1.868</td>
<td>2.603</td>
</tr>
<tr>
<td>80</td>
<td>2.381</td>
<td>1.396</td>
<td>1.325</td>
<td>1.804</td>
</tr>
</tbody>
</table>
is no response to the point of envisaging a possible blockage of the metabolic activity in progress. Thus, Kadri and Midoun (2015), justify the reduction in proline content in some species under stress by their ability to provide osmotic adjustment using other osmoregulators.

The proline content recorded in *Raphanus sativus* L. under saline stress rises gradually with increasing saline doses. These results are compatible with several studies on various species, namely *Brassica juncea* (Mittal et al., 2012), *Portulaca aleracea* (Rahdari et al., 2012) and *Phaseolus vulgaris* (Tahri, 2018). The accumulation of proline in response to saline stress may be due to increased its biosynthesis, decreased their catabolism, or their combination (Trinchant et al., 2004).

**Effect of copper-salinity interaction on total soluble sugars content**

The increase in Cu doses causes a progressive decrease in the soluble sugars content in the both parts of Radish compared to that recorded in the control. Unlike Cu, NaCl and the Cu-NaCl interaction cause an increase in soluble sugars levels in the aerial and root system of plant (Fig. 2). The highest soluble sugars content of the aerial parts is recorded in the control and that of the root parts is marked at 1000 PPM of Cu combined with 40 Meq/L of NaCl (Table 3). However, the lowest sugars content in both aerial and root parts are recorded at 1000 PPM combined with 40 Meq/L and 800 PPM combined with 80 Meq/L interactions respectively (Table 3).

Statistically, the variation in soluble sugars contents of Radish treated with Cu and Cu-NaCl is highly significant for the both parts of plant (Pd<0.005). However, the effect of NaCl on soluble sugar levels is not significant in the aerial part (P>0.05), but highly significant in the root part of plant (Pd<0.005).

Soluble sugars decrease in the aerial and root parts of Radish as copper doses increase. This response is similar to that reported by (Choudhary et al., 2012 a,b), where the soluble sugars content of Radish was reduced by about 2.1 times in response to Cu stress and 2.80 times under Cr stress. Other studies also signal a reduction of soluble sugars levels in Radish under Zn effect (Vijayarengan, 2012) and under Co effect (Kaliyamoorthy et al., 2007). Various studies have reported the decrease in soluble sugars content in other species, namely: *Cyanopsis tetragonoloba* L. Taub (Manivasagaperumal et al., 2011) and the leaves of *Triticum aestivum* under Zn action (Kumar et al., 2012), *Phaseolus vulgaris* under Cd and Pb effect (Bhardwaj et al., 2009). This decrease is caused by copper toxicity since it manifests mainly through rhizotoxicity (Kabata-Pendias and Pendias, 1992; Marschner, 1995), because they reduce the transport of sugars and carbohydrates to growing cells and inhibit amylases and phosphatases (Mihoub et al., 2005). According to (Rosa et al., 2009), salinity and low temperatures increase soluble sugars concentrations and heavy metals in general reduce it.

The results obtained reveal a gradual increase in soluble sugars content as doses of NaCl rise. These results are consistent with those obtained in *Cucurbita pepo* (Ialelou et al., 2013), *Medicago sativa* (Kadri and Midoun, 2015), for which an increase in soluble sugars is recorded as salt doses rise. According to (Ialelou et al., 2013), the increase in sugars has been linked to the degradation of starch and (Subba et al., 2014), justify this increase by the disruption of photosynthetic activity which could modify the metabolism of sugars.

**Effect of salinity on the tolerance of *Raphanus sativus* L. to copper stress**

The results obtained show that proline and soluble sugars levels in Radish treated with Cu-NaCl increase compared to those recorded under Cu effect alone. It appears that salinity promotes the tolerance of Radish against copper by increasing the synthesis of Proline and soluble sugars; in order to maintain its osmotic adjustment.
The Cu-NaCl interaction revealed a positive effect of salinity on the increase in proline and soluble sugars levels in the aerial part of Radish compared to the effect of copper alone on these levels. This indicates that salinity favours copper phytoextraction and their passage to the aerial part of Radish. These results are similar to those obtained by (Azzouz, 2011), showing that Vicia faba accumulate soluble sugars and proline under the influence of lead-NaCl interaction in an excessive manner compared to their accumulation under stress of lead and NaCl separately. Thus, this accumulation remains important in the aerial part that the root part of Vicia faba. According to (Valentina et al., 2010), it is not totally unexpected that salinity exercised a positive effect on the plants’ tolerance to metal stress. By contrast, significant data indicating a reduction or neutralization of heavy metal toxicity and an improvement in plant condition are presented in a few reports only, (Volkov et al., 2006; Ghnaya et al., 2007).

Conclusion

The Radish reduces its proline and sugars levels under copper stress and increases these levels under NaCl and Copper-NaCl interaction stress, this means that salinity has a positive action on the tolerance of plants to copper and it can increase the level of these osmoregulators to maintain its osmotic adjustment. The content of proline and soluble sugars is higher in root part under Cooper stress. By contrast, the addition of NaCl to copper increases this content in the arial part, which shows that the copper level rises in this part. The results obtained show that moderate salinity can improve the tolerance of Raphanus sativus L. to copper quite effectively, which should be taken into account during the development of phytoremediation technologies.

References


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